THE INFLUENCE OF PIROMEN ON THE REGENERATION OF THE SPINAL CORD OF THE YOUNG ADULT LIZARD, ANOLIS CAROLINENSIS, AFTER TRANSECTION

A THESIS

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CHAPTER I

INTRODUCTION

White ('25) studied the anatomical and histological aspects of the lizard's tail after it had undergone autotomy. He found that the spinal cord was incapable of regeneration. The cord was represented by a sheath of epithelial cells. This conclusion was due primarily to the staining technique employed. Kamrin and Singer ('55) studied the importance of the spinal cord in tail regeneration after it had been mechanically transected. They found that only limited regeneration of the tail occurred following the destruction of the spinal cord.

There has also been a use of drugs in the study of regeneration. One of these drugs is piromen which is an extract of the protozoan, Pseudomonas. According to McCullough ('59), piromen retards the fibrosis of the wound area and keeps the scar vascularized until the regenerative process is complete. Drummond ('54) observed regeneration of the spinal cord in the amphibian, Triturus viridescens, under the influence of piromen. He noted that the animals treated with piromen underwent structural regeneration in 60 days. Animals which did not receive the drug did not reveal structural regeneration until a later date. This study was made to ascertain the influence piromen may have on regeneration in one of the reptiles.

CHAPTER II

REVIEW OF LITERATURE

White ('25) observed tail regeneration of the adult Lizard after it had undergone a normal process of autotomy. The process of autotomy was instantaneous and left a clean edge. It occurred slightly beyond the cloacal opening, posterior to the strong lateral muscles attached to the body of the anterior caudal vertebra. In all of the animals, the fracture occurred through the middle of the same vertebral body. After the old tail had been discarded, a new one grew from the stump. It often reached the full size of the original tail and served as a functional structure. Histological sections were made of the regenerating tail at different intervals. Early observations of transverse and longitudinal sections revealed that at the site of autotomy the spinal cord diminished in size until it was reduced to a sheath of epithelial cells and fibers which surrounded the central canal. The lumen of the tube was occupied by connective tissue which contained numerous blood vessels and pigmented cells. This tube could be traced to the actual tip of the tail. There was no regeneration of the cord itself.

It was observed in the fully regenerated tail that a tube of cartilage replaced the vertebral column. In place of the spinal cord there was a canal lined with epithelium which resembled that which lines the canal of the intact cord. A mass of fatty connective tissue surrounded the cartilaginous tube. Nerve and blood elements revealed abnormal growth, but there was no impairment of function. Skin and muscle development was normal. It was concluded that the spinal cord was represented by a simple epithelial tube which resembled the filum terminale. The skeleton was represented by a continuous

tube of cartilage.

Kamrin and Singer ('55) investigated the importance of the spinal cord for regeneration of the tail of Anolis carolinensis. A portion of the spinal cord was destroyed by reaming it out for a distance of one centimeter with a thin wire. Eight organisms served as experimentals and 6 served as controls. The spinal canal of the experimentals was filled with paraffin or bone wax. The animals were sacrificed 121 to 184 days post-operatively. Regeneration in the controls was typical of that which occurs in most vertebrates. In the experimental animals only limited regeneration of the tail occurred. It was concluded that no significant regeneration of the lizard's tail occurred after the spinal cord near the wound area was destroyed.

Piatt ('55) transected the spinal cord in the region of the trunk of the adult Japanese newt, Triturus pyrrhogaster, in order to investigate regeneration. The animals were observed over a period of 175 days post-operatively. Histological sections were made of the regenerating cord. Observations of the sections of the 7th day regenerates revealed that the gap was filled with blood cells and debris of nerve elements. There was no sign of mesodermal connective scar tissue or regenerating fibers extending from the cut ends of the cord. On the 16th day, many fibers had migrated into the cut area, and on the 20th day, the fibers ended in a terminal compact club. The terminal portion of the fibers revealed degenerative changes. From the 30th day to the last day on which the animals were sacrificed regeneration consisted chiefly in the large number of nerve fibers which grew from both ends of the cord into the region of the transection. The older regenerated cords did not attain a normal diameter through the region of the cut, but the outer white matter of the cord was normal in appearance. Mauthner's fibers did not

regenerate. It was concluded that complete transection was followed by anatomical repair.

Smith ('58) studied regeneration of the spinal cord of the adult Triturus viridescens. The spinal cord was completely transected in the cervical region between spinal segments one and 4. He found that the cord was capable of extensive morphological and histological regeneration after transection. One important factor which seemed to have bearing on the extent and rate of regeneration was the amount of space left between the cut ends of the cord. According to Smith, the spinal cord in this experiment was transected without the removal of the entire segment. This may have contributed to the rate of regeneration and extensive morphological restoration which took place in the transected cord.

Windle and Chambers ('50) transected the spinal cord of adult cats and dogs to study the site of action of injected bacterial pyrogenic extracts. Dosages ranged from 300 ug. per kg. to 11,000 ug. per kg. body weight. The controls were given no type of drug to aid regeneration. In animals which did not receive pyrogenic extracts scars which consisted of collagenous connective tissue were present. Nerve fibers were not observed in the area of the scar. In animals that were given the extracts, scar tissue did not appear. The matrix consisted of loose tissue which resembled macrophage and reticular cells. Small blood vessels were present in the loose tissue. It was concluded that limited anatomical regeneration can take place in the presence of pirogen.

Windle, Clemente and Chambers ('52) studied the effects of bacterial polysaccharides on a peripheral nerve growing into the brain. The right temporal branch of the facial nerve was cut at its distal end in two cats.

The cut end of the nerve was inserted through the meninges into the right temporal lobe of the cerebrum to a depth of three to 4 mm. One animal was given Pyromen R intravenously every other day for 28 days. Dosages ranged from 10 ug. per kg. to 18 ug. per kg. body weight. The control received no type of drug to aid regeneration. The animals were sacrificed on the 36th day after the operation. Histological sections were made of the implants.

A study of the sections through the implants of the control revealed that a glial barrier was present. The glial barrier varied from a few to many cells in thickness. The regenerating nerve fibers grew along the neurolemma, but upon reaching the barrier they turned back into the center of the nerve. Thus, blending of the nerve with the brain tissue did not occur. Observations of sections of the nerve implant in the piromen treated animal revealed that regenerating nerve fibers, neurolemma cells, connective tissue and blood vessels had grown into the brain. It was concluded that the nerve in the animal treated with piromen readily grew into the brain.

Arteta ('56) studied the effects of pyrogens on the regeneration of the spinal cord in cats. They were placed under ether anesthesia and a laminectomy was performed in the dorso-lumbar region. After having exposed the spinal cord, an incomplete transverse section was made in all of the experimental animals in order to leave a bridge of nerve elements. Five organisms served as experimentals and 4 served as controls. From the third day after the operation, the experimental animals were given the pyrogenous solution by intravenous injections. The animals were sacrificed on the 64th day after the operation. Histological sections were made to study regeneration.

In the control animals, a cicatrix was discovered at the level of the hemisection. The experimental animals had a larger cicatrix of greater and

looser limits than did the controls. He concluded that in the animals subjected to the action of the bacterial pyrogen solution, a modification of the medullary cicatrix occurred. As a consequence, the cicatrix was less dense, had a looser structure and the cicatricial area was apparently better vascularized; it's limits were marked out less clearly than those of the controls. This was due to the invasion of the adjoining nerve elements by sprouts of vascular connective tissue which preceeded from the central cicatrix and advanced between the nerve bundles of the cord. The greater looseness of the newly-formed tissue brought about the formation of abundant and more complicated plasmatic cysts, and were obstacles to the regenerative process.

Arteta ('56) studied the influence of pyrogenous substances on the regeneration of a peripheral nerve. The proximal end of the hypoglossal nerve was transected in 13 cats. Six served as experimentals and 7 served as controls. The experimentals were injected with daily dosages of the pyrogenous bacterial polysaccharide extract. The animals were sacrificed 20 days after the operation. Histological sections were made of the transected nerve. In animals treated with piromen the cicatricial neuromas were more exuberant and more adherent to the adjoining tissue than the ones in the controls. Regeneration in both the control and experimental animals was of the heterogenic type as revealed by similar histological results.

McCullough ('59) studied the effects of piromen on scar formation of mesodermal origin at the site of injury of peripheral nerves. Ten rats served as controls and 10 served as experimentals. Seventy-five to 90% of the sciatic nerve was cut through at the thigh. The experimentals were given dosages of piromen which varied from 0.25 ug. per kg. to 25.0 ug. per kg. body weight.

Histological sections were made of the regenerating fibers at various intervals. Observations of the sections of the 5th day, in both control and experimental animals, revealed that the wound site was highly cellular. It also contained a fine, loose stroma of reticular fibers and was richly vascularized. Observations of the sections of control and experimental animals on the 9th day revealed similar results. Fibroblasts were numerous and the matrix of the scars was looser and denser in the controls than that found in the experimental animals. In the animals sacrificed at intervals of from 120, 150, and 180 days after surgery, neuromata marked the site of injury in the controls. There was little or no sign of injury in the experimental animals at the same period of regeneration. It was concluded that piromen retarded the collagenous fibrosis of scars and kept the scar loose under the conditions of this experiment.

CHAPTER III

MATERIALS AND METHODS

The animals used in this experiment were young adult Anolis carolinensis obtained from Turtox Biological Supply Company, Chicago, Illinois, and Carolina Biological Supply Company, Elon College, North Carolina. They measured from 8 to 12 centimeters in length. The stock animals were kept in a rectangular terrarium which contained vegetation similar to that of the normal habitat. They were fed meal worms cut into small pieces.

The animals were immobilized by the use of ether after which a complete transection of the spinal cord was made in the sacral region between segments one and 4. All of the incisions were made with iridectomy scissors which had been sterilized with ethyl alcohol. Care was taken not to cut or injure the dorsal aorta.

Immediately after the operation, the animals were placed in sterilized finger bowls which had been exposed to dry heat for one hour at 150°C. A total of 135 organisms was used in this investigation. Forty-five served as experimentals and 42 served as controls. The experimental animals were injected weekly with two-tenths millimeters of piromen, while the controls were not administered any type of injection. The lizards were sacrificed three, 4, 6, 7, 15, 16, 20, 23, 24, and 30 days after the operation. A portion of the vertebral column, one to two millimeters on each side of the cut region, was removed. The tissue was then placed in a fixative, suggested by Bodian, for from 5 to 6 days. At the end of this period, it was placed in decalcifying solution for 20 days. It was then dehydrated, infiltrated, embedded and longitudinal sections of 10 μ were made. Sections were stained by the Bodian-protargol or Pyridine-silver methods.

CHAPTER IV

EXPERIMENTAL RESULTS

The following data was compiled from an investigation of the spinal cord of young adult lizards, Anolis carolinensis, after transection.

Description of the Intacr Cord

The gray and white matter are marked out distinctly in the spinal cord. The fibers of the latter follow an irregular course in and out of the gray matter and are located within the outer region of the spinal cord. The gray matter is composed of numerous nerve cell bodies located around the neural canal. Ependymal cells line the central canal (figs. 1 and 2).

The Regenerating Cord

In the early stages of regeneration in the control large nucleated cells were present throughout the distal end of the transection, while the proximal end was similar to the intact cord. A gap separated the two ends of the cord and there was no evidence of repair (fig. 3). Large nucleated cells were not located around the wound of the experimental cord, however, some fibers were located in the gap between the two ends left by the transection (fig. 4).

Masses of scar tissue were located throughout the cut area in the control cord during the second stage of regeneration. Large nucleated cells were neither found in this stage, nor was the gap present in the injured portion of the cord (fig. 5). The experimental cord at this stage was histologically similar to that of the intact cord. There were only limited amounts of scar tissue present in the surrounding area at the site of the wound. The tissue had a loose appearance and was vascularized (fig. 6).

Masses of scar tissue were present at the site of injury and in the surrounding tissue during the third stage of regeneration in the control cord (fig. 7), while a similar condition was not present in the experimental cord at this stage. The arrangement of gray and white matter was similar to that of the intact cord. A few white blood cells were sparsely located throughout the site of injury (fig. 8).

Fibers were observed across the cut area of the regenerating cord during the latter stages of the investigation. Close observations revealed distinct looseness of the tissue (figs. 9 and 10).

CHAPTER V

DISCUSSION

Results observed in this investigation revealed evidence that the spinal cord of young adult lizards, Anolis carolinensis, is capable of undergoing extensive histological and morphological regeneration after the cord is completely transected. Regeneration occurred along the same lines as those of other higher vertebrates.

Organisms treated with piromen revealed marked histological differences in comparison to untreated animals during the early phases of the process. The cut region remained loose and vascular in the experimental cords, while the controls contained masses of scar tissue which seemed to act as an obstacle to the regenerative process. These dense masses of scar tissue were present at the site of injury and surrounding the area of the wound. These masses of scar tissue were present up to the latter stages of regeneration. These reactions were similar to the experimental tissue observed by McCullough ('59) who studied the regenerative ability of the sciatic nerve transected at the level of the thigh in adult rats. He found that the scar tissue present in the controls served as a hindrance to regeneration. The work of Windle et. al ('52) revealed that piromen keeps the scar loose and vascular during the early stages of regeneration. These observations were noted in the present investigation. The regenerating nerve tissue of the experimentals remained loose throughout the entire period of the investigation.

During the early stages of regeneration, the control cord possessed large nucleated cells which contained chromidial material. Only a few nucleated cells were visible in the experimental cord. However, the control cord was able to undergo limited repair.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. The spinal cord of young adult lizards, Anolis carolinensis, was transected at the level of the sacral region and histological and morphological observations were made on the process of regeneration for 30 days.

2. Control and experimental animals were used. The former were untreated and the latter were injected with piromen.

3. Large masses of scar tissue were located throughout the cut area in the control cord during the early phases of the investigation.

4. The wound area of the experimental animals remained vascular and highly cellular throughout the regenerative process.

5. The spinal cord of young adult Anolis carolinensis has the ability to undergo limited histological and morphological regeneration following transection.

6. It may be concluded that piromen keeps the wound area loose which may serve as an aid to morphological repair.

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PLATE I

(Explanation of Figures)*

*All figures are photomicrographs of stained sections

(Explanation of Figures)

Fig. 1. Sagittal section through the unoperated cord showing gray and white matter, nerve cell bodies and longitudinally arranged fibers of the white matter. X 100.

Fig. 2. Cross section of the unoperated cord showing white and gray matter and ependymal cells which line the central canal. X 100.

Fig. 3. Sagittal section of the spinal cord of a three day control showing large chromidial cells located in the distal end of the cord. A gap separates the two ends of the cord. X 100.

Fig. 4. Sagittal section of the spinal cord of a 4 day experimental showing fibers between the two ends of the cord left by the transection. X 100.

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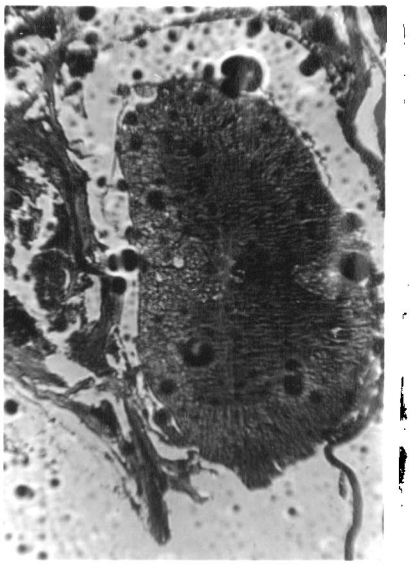

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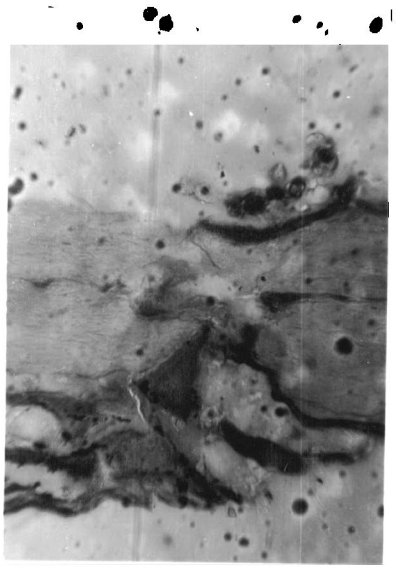

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PLATE II

(Explanation of Figures)*

*
All figures are photomicrographs of stained sections.

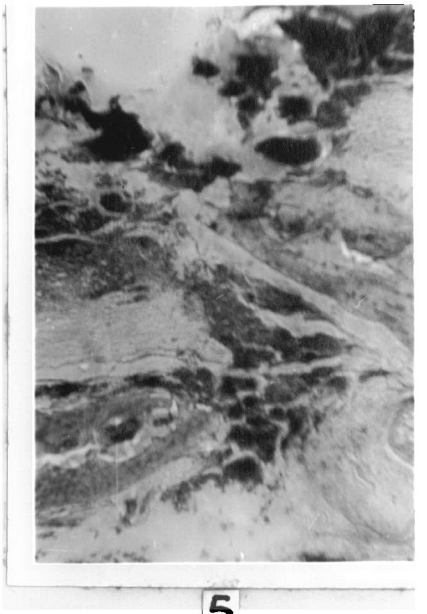
(Explanation of Figures)

Fig. 5. Sagittal section of the spinal cord of a 7 day control showing masses of scar tissue in the cut area and surrounding tissue. X 100.

Fig. 6. Sagittal section of the spinal cord of a 6 day experimental showing limited amounts of scar tissue and loose appearance of the tissue. X 100.

Fig. 7. Sagittal section of the spinal cord of a 16 day control showing masses of scar tissue present at the area of the wound. X 100.

Fig. 8. Sagittal section of the spinal cord of a 15 day experimental showing distinct looseness of the nerve tissue and white blood cells present in the cut area. X 100.



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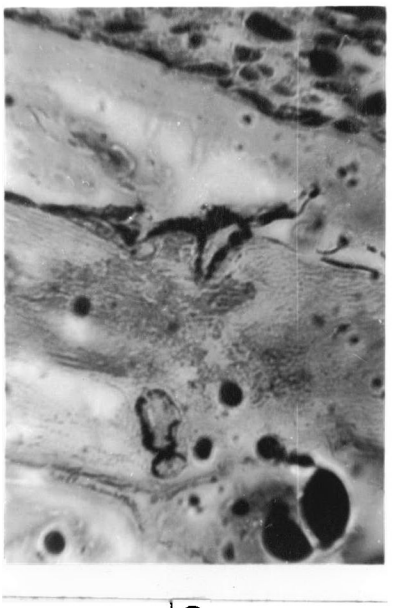

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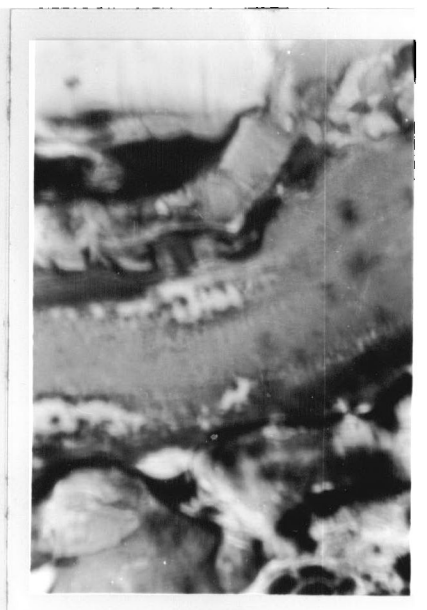

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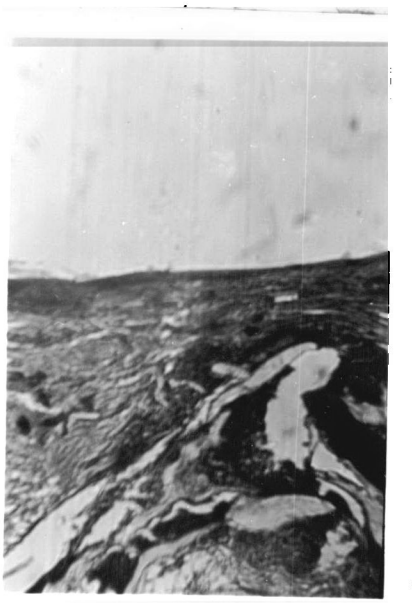

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PLATE III

(Explanation of Figures)*

*All figures are photomicrographs of stained sections.

(Explanation of Figures)

Fig. 9. Sagittal section of the spinal cord of a 30 day experimental showing fibers across the gap of the regenerating cord. X 100.

Fig. 10. Sagittal section of the spinal cord of a 30 day experimental showing loose and vascular appearance of the tissue. X 430.

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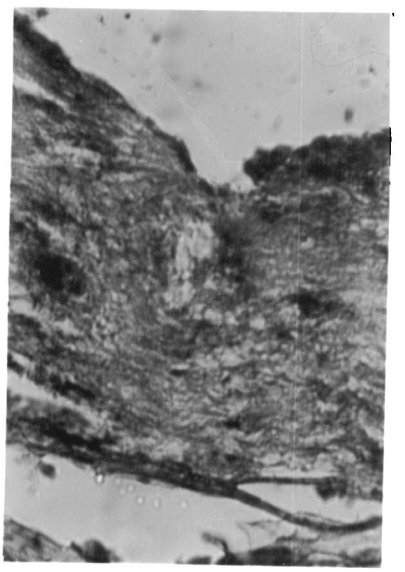

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